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GRANT NO: DAMD17-94-J-4186

TITLE: The Genetic Analysis of the Role of NEU Differentiation Factor (Heregulin) in NEU-Induced Mammary Carcinomas in the Mouse

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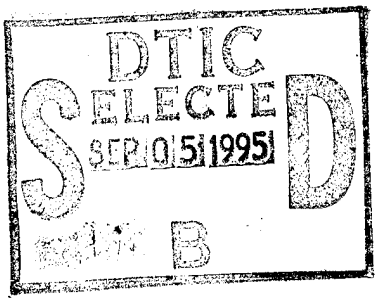


REPORT DATE: July 13, 1995

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

Form Approved
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| 1. AGENCY USE ONLY (Leave blank) | 2 13 July 1995 | 3. REPORT TYPE AND DATES COVERED Annual 15 Jul 94 - 14 Jun 95 |
|---|---|---|

**4. TITLE AND SUBTITLE**
The Genetic Analysis of the Role of Neu Differentiation Factor (Heregulin) in Neu-Induced Mammary Carcinomas in the Mouse

**5. FUNDING NUMBERS**
DAMD17-94-J-4186

**6. AUTHOR(S)**
Dr. Archibald Perkins

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
Yale University School of Medicine
New Haven, Connecticut 06520-8047

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSORING/MONITORING AGENCY REPORT NUMBER**

**11. SUPPLEMENTARY NOTES**

**12a. DISTRIBUTION/AVAILABILITY STATEMENT**
Approved for public release, distribution unlimited

**12b. DISTRIBUTION CODE**

**13. ABSTRACT** *(Maximum 200 words)*

**Utilization of the mouse to investigate the genetics of mammary carcinoma.** Identification of cooperating oncogenes using MMTV-insertion mutagenesis. It is known that over-expression of normal *neu* in the breast can lead to an increase in mammary carcinomas in transgenic mice, but the tumors occur late, indicating that additional mutations are necessary. One can target these cooperating genes by insertional activation with MMTV. The isolation and characterization of activated genes by molecular cloning techniques may lead to the identification of novel oncogenes involved in murine mammary tumors, and perhaps analogous human tumors as well.

Although the ligand heregulin may not bind directly to the $p185^{neu}$ receptor, they have been shown to activate neu kinase activity. Tumorigenesis via $p185^{neu}$ signaling pathways could occur via ligand dependent or ligand independent routes. The relative importance of these two route may be determined genetically. We plan to test for cooperativity between *neu* and heregulin by creating transgenic mice that overexpress heregulin and cross these to *neu* transgenics. A dramatic increase in tumor incidence in neu transgenic mice bearing a heregulin transgene would indicate an important role for this ligand in the process.

| 14. SUBJECT TERMS | | 15. NUMBER OF PAGES |
|---|---|---|
| heregulin<br>transgenic mice | oncogene cooperation | 7<br>16. PRICE CODE |

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
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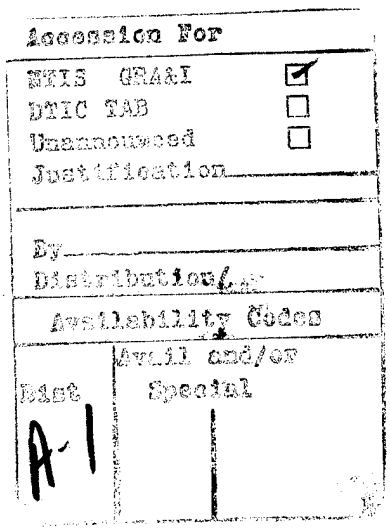


Archibald S. Perkins MD PhD 7/13/95
PI - Signature Date

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# INTRODUCTION

Like most human cancer, breast cancer is the result of cumulative genetic alterations resulting in loss of growth control. The genes involved in this multistep process have not bee elucidated, but include p53, which is altered in more than 50% of cases, the Rb tumor suppressor gene, BRCA1 (5% of cases, mostly inherited, early onset), and the *neu/erbB-2/HER-2* gene in 20-30% of cases(Slamon, Clark, Wong, Levin, Ullrich, & McGuire, 1987); it is this last gene, *neu*, which is the focus of our research. *neu* is a member of a family of genes that encode receptor tyrosine kinases. Other family members include the epidermal growth factor receptor (*EGFR*), *erbB-3*, and *erbB-4*. Activated *neu* oncogenes are potent in transforming cells in culture and transgenic mice overexpressing either mutationally activated *or* normal *neu* in the mammary gland succumb to adenocarcinomas. The oncogenic effect of both activated and normal neu alleles was evident from whole animal studies (Muller, Sinn, Pattengale, Wallace, & Leder, 1988). When normal c-*neu* gene was driven by MMTV in transgenic animals, the tumors were focal adenocarcinomas surrounded by hyperplasia, and were not pregnancy dependent(Guy, Webster, Schaller, Parsons, Cardiff, & Muller, 1992). Since the mode of c-*neu* participation in oncogenesis in humans is amplification rather than activating mutations at c-*neu*, this transgenic model more closely resembles the situation in humans, and the long latency and stochastic nature of the tumors emphasizes the need for other events in carcinogenesis.

The $p185^{neu}$ receptor encoded by *neu* is stimulated by two families of ligands: the EGF family, and the NDF family, which includes heregulin(Holmes, 1992), also known as neu differentiation factor (NDF)(Wen, Peles, Cupples, Suggs, Bacus, Luo, et al., 1992). None of the EGF family members appears to bind directly to $p185^{neu}$, yet several can activate the receptor via transmodulation. This is believed to occur by binding of the ligand to a high affinity receptor (e.g., EGFR) which then physically associates with $p185^{neu}$ and heterodimerizes. The result of this physical association is phosphorylation and activation of $p185^{neu}$. Thus a variety of ligands can channel their signal through $p185^{neu}$, and the partners created depend on what other receptors are expressed in a given cell, and what ligands the cell is exposed to.

NDF is synthesized initially as a transmembrane glycoprotein with a 242 amino acid ectodomain that has an IgG-type motif and an EGF homology domian. The latter, contained in all members of the ErbB-binding ligand family, most likely functions in receptor binding. The transmembrane form, via proteolysisis at a site near the ecto-/transmembrane domain junction, is likely to be the precursor for the released form, as is the case for other membrane-bound growth factors.

While little is known about the role of NDF in mammary carcinogenesis, the role of other ligands that act through ErbB family members in malignancy has been investigated. Transgenic mice overexpressing TGFa, either with promoters targeting mammary epithelium, or generalized promoters, display mammary epithelial hyperplasia (Matsui, Halter, Holt, Hogan, & Coffey, 1990) and neoplasia that is often malignant, and often involves the terminal ducts and secretory alveoli. Recent studies show a potent interaction between TGFa and c-*neu* overexpression in transgenic mice: By crossing the MMTV-c-*neu* transgenics with MMTV-TGFa mice, a strong cooperativity was found, resulting in rapid hyperplasia and milk production (Muller, pers. comm). Clearly TGFa has a mitogenic, growth-stimulatory role in breast development and in mammary carcinogenesis. The role of NDFs is unclear, but given the finding that it can promote differentiation and growth cessation in cultured mammary epithelial cells, it may act antagonistically to TGFa. It is the goal of these studies to explore the role of NDFs in mammary carcinogenesis in whole animals using genetic approaches.

## BODY

**Task 1. Overexpression of NDF: Transgenics** *neu* is one of the few genes clearly implicated in the development of human mammary tumors. In addition, TGFα, a ligand for a related growth factor receptor, EGFR, can stimulate $p185^{neu}$ activity via transmodulation, and can also play a stimulatory role in mammary carcinogenesis. We hypothesize that NDF, a putative ligand for $p185^{neu}$ that can stimulate its activity, but which induces the differentiation of mammary cell lines, plays an important role in mammary tumors, especially those in which *neu* also has a causative role. To address this hypothesis, we propose to direct overexpression of NDF to the mammary gland of transgenic mice, and to see what effect this has on mammary growth and development, as well as susceptibility to c-*neu*-induced or MMTV-induced mammary tumors in mice. To this end, we have so far made transgenic mice with NDF under transcriptional control of MMTV (14 founders) and under control of the whey acidic promoter (18 founders). These are being analyzed for expression

Task 2. Targeted deletion of NDF via homologous recombination. In these experiments, we take another approach to testing the same hypothesis that NDF plays an important role in mammary development and neoplasia. If this hypothesis is true, then deletion of the gene encoding NDF should have effects on either mammary gland development or neoplasia, or both. This will be accomplished by targeted deletion of the gene in mice via homologous recombination in embryonic stem (ES) cells. The null allele generated will be made homozygous, and then put onto several genetic backgrounds to assess the effect of this mutation on both normal gland development, and on MMTV-c*neu*-induced mammary carcinomas. We plan to embark on these studies in the coming year.

Task 4. Identification of protooncogenes that can cooperate with *neu*. It is clear from the studies of Muller and coworkers that *neu* does not act alone in the generation of mammary carcinomas in transgenic mice. The long latency (5-8 months) and the solitary, stochastic nature of the tumors argues that other factors are necessary in the disease process. We thus hypothesize that while *neu* is an important oncogene in mammary tumorigenesis, other genes are involved, and we propose to identify what these other genetic factors are by retroviral mutagenesis and proviral tagging. This is being accomplished by infection of transgenic MMTV-c*neu* mice with mouse mammary tumor virus. We expect that infection of transgene with the virus will cause an acceleration of tumorigenesis: a shortening of tumor latency, due to the activation of cellular genes that can cooperate with c*neu* in the development of tumors. The presence of the proviral tag in *cis* to the implicated oncogene will enable us to molecularly clone and characterize them. This approach will allow us to find out what other genes need to be altered, and if the function of these genes can be discerned, what other aspects of cellular growth control must be deregulated, in order to arrive at a fully malignant cell. To perform the experiment correctly, we are backcrossing the MMTV-c*neu* transgene onto the C3H background for five generations, so that the genetic background will be essentially identical to C3H, the high mammary carcinoma strain that carries MMTV. We are almost completed with this phase of the project, and will begin to age mice that have both the transgene and MMTV, to look for acceleration of tumorigenesis.

## CONCLUSIONS

The projects are progressing as scheduled, and in the coming year they should begin to yield results.

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